The array was composed of oligonucleotide probes (25-30 mer) complementary to four virulence loci (intimin, Shiga-like toxins I and II, and hemolysin A). Target DNA was amplified from whole cells or from purified DNA via single or multiplexed polymerase chain reaction (PCR), and PCR products were hybridized to the array without further modification or purification. The array was 32-fold more sensitive than gel electrophoresis and capable of detecting amplification products from <1 cell equivalent of genomic DNA (1 fg). Immunomagnetic capture, PCR and a microarray were subsequently used to detect 55 CFU ml-1 (E. coli O157:H7) from chicken rinsate without the aid of pre-enrichment. Four isolates of E. coli O157:H7 and one isolate of O91:H2, for which genotypic data were available, were unambiguously genotyped with this array. Glass-based microarrays are relatively simple to construct and provide a rapid and sensitive means to detect multiplexed PCR products; the system is amenable to automation.

L15 ANSWER 74 OF 91 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:369872 BIOSIS
DOCUMENT NUMBER: PREV200000369872
TITLE: Detecting and genotyping pathogens using low-density microarrays.
AUTHOR(S): Call, D. R. [Reprint author]; Chandler, D. P. [Reprint author]; Brown, J. D. [Reprint author]; Stottlemyer, M. [Reprint author]; Jutras, E. M. [Reprint author]; Brockman, F. J. [Reprint author]
CORPORATE SOURCE: Pacific Northwest National Laboratory, Richland, WA, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2000) Vol. 100, pp. 168. print.
Meeting Info.: 100th General Meeting of the American Society for Microbiology. Los Angeles, California, USA. May 21-25, 2000. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 Aug 2000
Last Updated on STN: 8 Jan 2002

=> d hist

(FILE 'HOME' ENTERED AT 10:58:59 ON 24 JUN 2007)

FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 10:59:19 ON 24 JUN 2007

L1 60 S ("FRET") AND (PATHOGEN) AND (FUNGAL OR (GRAM(W)NEGATIVE) OR (
L2 91 S ("FRET") AND (PATHOGEN) AND PROBE?
L3 425 S PATHOGEN AND PROBE? AND MULTIPLEX?
L4 148 S L3 AND (FUNGAL OR (GRAM(W)NEGATIVE OR GRAM(W)POSITIVE))
L5 232 S PATHOGEN? AND PROBE? AND (LIGHTCYCLER)
L6 52 DUP REM L1 (8 DUPLICATES REMOVED)
L7 79 DUP REM L2 (12 DUPLICATES REMOVED)
L8 326 DUP REM L3 (99 DUPLICATES REMOVED)
L9 137 DUP REM L4 (11 DUPLICATES REMOVED)
L10 184 DUP REM L5 (48 DUPLICATES REMOVED)
L11 24 S L1 AND PY<=2003
L12 24 S L6 AND PY<=2003
L13 47 S L7 AND PY<=2003
L14 170 S L8 AND PY<=2003
L15 91 S L9 AND PY<=2003
L16 117 S L10 AND PY<=2003

```
=> d hist full

     (FILE 'HOME' ENTERED AT 10:58:59 ON 24 JUN 2007)

     FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 10:59:19 ON 24 JUN 2007
L1           60 SEA ABB=ON  PLU=ON  ("FRET") AND (PATHOGEN) AND (FUNGAL OR
                (GRAM(W) NEGATIVE) OR (GRAM(W) POSITIVE))
L2           91 SEA ABB=ON  PLU=ON  ("FRET") AND (PATHOGEN) AND PROBE?
L3          425 SEA ABB=ON  PLU=ON  PATHOGEN AND PROBE? AND MULTIPLEX?
L4          148 SEA ABB=ON  PLU=ON  L3 AND (FUNGAL OR (GRAM(W) NEGATIVE OR
                GRAM(W) POSITIVE))
L5          232 SEA ABB=ON  PLU=ON  PATHOGEN? AND PROBE? AND (LIGHTCYCLER)
L6           52 DUP REM L1 (8 DUPLICATES REMOVED)
L7           79 DUP REM L2 (12 DUPLICATES REMOVED)
L8          326 DUP REM L3 (99 DUPLICATES REMOVED)
L9          137 DUP REM L4 (11 DUPLICATES REMOVED)
L10         184 DUP REM L5 (48 DUPLICATES REMOVED)
L11          24 SEA ABB=ON  PLU=ON  L1 AND PY<=2003
L12          24 SEA ABB=ON  PLU=ON  L6 AND PY<=2003
L13          47 SEA ABB=ON  PLU=ON  L7 AND PY<=2003
L14         170 SEA ABB=ON  PLU=ON  L8 AND PY<=2003
L15          91 SEA ABB=ON  PLU=ON  L9 AND PY<=2003
L16         117 SEA ABB=ON  PLU=ON  L10 AND PY<=2003
                D L12 TI 1-24
                D L13 TI 1-25
                D L13 TI 26-47
                D L15 TI 1-30
                D L15 TI 31-60
                D L15 TI 61-91
                D L12 IBIB ABS 1,3-5,10,13,16,21,23
                D L13 IBIB ABS 3,6,8,9,27,33,41,46
                D L15 IBIB ABS 5,8,18,22,40,51,65,74


     FILE HOME

     FILE MEDLINE
      FILE LAST UPDATED: 23 Jun 2007 (20070623/UP).  FILE COVERS 1950 TO DATE.

        This file contains CAS Registry Numbers for easy and accurate
        substance identification.

     FILE CAPLUS

     Copyright of the articles to which records in this database refer is
     held by the publishers listed in the PUBLISHER (PB) field (available
     for records published or updated in Chemical Abstracts after December
     26, 1996), unless otherwise indicated in the original publications.
     The CA Lexicon is the copyrighted intellectual property of the
     American Chemical Society and is provided to assist you in searching
     databases on STN.  Any dissemination, distribution, copying, or storing
     of this information, without the prior written consent of CAS, is
     strictly prohibited.

     FILE COVERS 1907 - 24 Jun 2007  VOL 147 ISS 1
     FILE LAST UPDATED: 22 Jun 2007  (20070622/ED)

     Effective October 17, 2005, revised CAS Information Use Policies apply.
     They are available for your review at:

     http://www.cas.org/infopolicy.html
```

About Entrez
Text Version
Entrez PubMed
Overview
Help | FAQ
Tutorials
New/Noteworthy
E-Utilities
PubMed Services
Journals Database
MeSH Database
Single Citation Matcher
Batch Citation Matcher
Clinical Queries
Special Queries
LinkOut
My NCBI
Related Resources
Order Documents
NLM Mobile
NLM Catalog
NLM Gateway
TOXNET
Consumer Health
Clinical Alerts
ClinicalTrials.gov
PubMed Central

Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**

- Search History will be lost after eight hours of inactivity.
- Search numbers may not be continuous; all searches are represented.
- To save search indefinitely, click query # and select Save in My NCBI.
- To combine searches use #search, e.g., #2 AND #3 or click query # for more options.

Search	Most Recent Queries	Time	Result
#18	Search **(FRET) and (fungal or fungus or gram (negative or positive))** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	20:24:41	4
#17	Search **pathogen and (FRET) and (fungal or fungus or gram (negative or positive))** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	20:24:21	0
#16	Search **pathogen and (FRET and probe) and (fungal or fungus or gram (negative or positive))** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	20:24:10	0
#12	Search **pathogen and ("Lightcycler")** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	20:09:52	4
#11	Search **pathogen and ("Lightcycler") and (fungal or gram (negative or positive))** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	20:09:39	0
#6	Search **pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))** Limits: **Publication Date to 2003/12, only items with links to full text, only items with links to free full text**	19:26:00	27
#7	Search **pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))** Limits: **Publication Date to 2004, only items with links to full text, only items with links to free full text**	18:10:23	35
#5	Search **pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))** Limits: **Publication Date to 2003/12**	18:08:26	68
#4	Search **pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))**	18:07:52	122

#3	Search **pathogen and (hybridization or "Lightcycler")) and (fungal or gram (negative or positive))**	18:07:38	122
#2	Search **(pathogen and (hybridization or "Lightcycler")) and (fungal or gram (negative or positive))**	18:07:25	122
#1	Search **(pathogen and (hybridization or "Lightcycler") and (fungal or gram (negative or positive))**	18:06:51	122

Clear History

Lowercase **"or"** was ignored. Try **"OR"** to search for either of two terms. [details]
The **"AND"** operator is unnecessary -- we include all search terms by default. [details]

Scholar **All articles** - Recent articles Results **1 - 10** of about **210** for (**FRET** and **pathogen** and **fungal** or **fungus** or (**gram negative**) or (**gram positive**)). (0.06 s

All Results
H Wernerus
B Beutler
H Duclohier
L Schena
P Singleton

Quantitative test for bacterial pathogens
EJ Baron, WD Engel, G Haberhausen, P Kaspar, AM ... - 2006 - freepatentsonline.com
... for amplifying all types of **fungal** ribosomal 18S ... and subsequent analysis using **FRET** hybridization probes ... is specific for said bacterial **pathogen** is indicative ...
Cached - Web Search

Method for the detection of pathogenic **gram positive** bacteria from the genera staphylococcus, ...
G Haberhausen, T Emrich, R Rossasu, G Jannes, DD ... - 2006 - freepatentsonline.com
... primers for amplifying all types of **fungal** ribosomal 18S ... of a **FRET** donor moiety and a **FRET** acceptor moiety ... species or strain of the respective **pathogen** that has ...
Cached - Web Search

Method for the detection of pathogenic **gram positive** bacteria selected from the genera ...
GDRDB HABERHAUSEN, TDRDC EMRICH, R ROSSAU, GE ... - EP Patent 1,426,447, 2004 - freepatentsonline.com
... primers for amplifying all types of **fungal** ribosomal 18S ... of a **FRET** donor moiety and a **FRET** acceptor moiety ... species or strain of the respective **pathogen** that has ...
Cached - Web Search

Innate immunity markers for rapid diagnosis of infectious diseases - all 2 versions »
PJ Dailey - 2003 - freepatentsonline.com
... resonance energy transfer assay "**FRET** assay"), reverse ... a "type of infectious **pathogen**" includes determining ... eg, a bacterial, yeast, viral or **fungal** infection. ...
Cached - Web Search

TLR 4-dependent lipopolysaccharide signalling in epithelial cells is independent of extracellular ... - all 4 versions »
F Backhed, S Normark, A Richter-Dahlfors - Cellular Microbiology, 2002 - Blackwell Synergy
... TLRs), which discriminate potential **pathogens** from self. ... protease activities initiated upon **fungal** infections, which ... et al., 2001) and **FRET** technology (Jiang ...
Cited by 7 - Related Articles - Web Search - BL Direct

Multiplex assay detection of pathogenic organisms - all 2 versions »
G Haberhausen, T Emrich, G Sagner, M Moczko, G ... - 2006 - freepatentsonline.com
... a **FRET** donor moiety and a **FRET** acceptor moiety ... of a predetermined group of pathogenic fungi, said group ... is used to detect and identify **fungal pathogens** such as ...
Cached - Web Search

Innate immune responses to microbial poisons: Discovery and function of the toll-like receptors - all 5 versions »
B Beutler - Annual Review of Pharmacology and Toxicology, 2003 - phyto.annualreviews.org
... that Toll protects flies against **fungal** infection (68 ... resonance energy transfer (**FRET**) analyses suggest ... small contribution to susceptibility for most **pathogens**. ...
Cited by 29 - Related Articles - Web Search - BL Direct

INDEXES TO VOLUME 11
S Index - ingentaconnect.com
... prolonged, and community-acquired **pathogens** in children ... 486 Fluorescence resonance energy transfer (**FRET**) PCR, real ... 248 see also specific fungi/**fungal** infections ...
Web Search

INDEXES TO VOLUME 11 Subject Index
ID Albicans, B Amphotericin, C Candida - Clinical Microbiology & Infection, 2005 - Blackwell Synergy
... prolonged, and community-acquired **pathogens** in children ... resonance energy transfer (**FRET**) PCR, real ... 248 see also specific fungi/**fungal** infections Ganciclovir ...
Web Search

The Function of Mlo, A **negative** Regulator of Defence, is conserved in Monocot and Dicot plants
C Consonni - 2005 - kups.ub.uni-koeln.de
... **FRET** fluorescence resonance energy transfer ... structures essential for the survival of the **pathogen**. ... glucans, chitins and proteins derived from **fungal** cell walls ...
Cited by 2 - Related Articles - View as HTML - Web Search - Library Search

Charge Transfer Complexes by R. Foster, Academic Press, 1969), the laser energy can be tuned to the corresponding energy of the charge-transfer wavelength and, thus, a specific desorption off the solid support can be initiated. Those skilled in the art will recognize that several combinations can serve this purpose and that the donor functionality can be either on the solid support or coupled to the nucleic acid molecule to be detected or vice versa.

In yet another approach, a reversible L–L' linkage can be generated by homolytically forming relatively stable radicals. Under the influence of the laser pulse, desorption (as discussed above) as well as ionization will take place at the radical position. Those skilled in the art will recognize that other organic radicals can be selected and that, in relation to the dissociation energies needed to homolytically cleave the bond between them, a corresponding laser wavelength can be selected (see e.g., *Reactive Molecules* by C. Wentrup, John Wiley & Sons, 1984).

When performing exonuclease sequencing using MALDI-TOF MS, a single stranded DNA molecule immobilized via its 5'-end to a solid support is unilaterally degraded with a 3'-processive exonuclease and the molecular weight of the degraded nucleotide is determined sequentially. Reverse Sanger sequencing reveals the nucleotide sequence of the immobilized DNA. By adding a selectively cleavable linker, not only can the mass of the free nucleotides be determined but also, upon removal of the nucleotides by washing, the mass of the remaining fragment can be detected by MALDI-TOF upon cleaving the DNA from the solid support. Using selectively cleavable linkers, such as the photocleavable and chemical cleavable linkers provided herein, this cleavage can be selected to occur during the ionization and volatizing steps of MALDI-TOF. The same rationale applies for a 5' immobilized strand of a double stranded DNA that is degraded while in a duplex. Likewise, this also applies when using a 5'-processive exonuclease and the DNA is immobilized through the 3'-end to the solid support.

As noted, at least three version of immobilization are contemplated herein: 1) the target nucleic acid is amplified or obtained (the target sequence or surrounding DNA sequence must be known to make primers to amplify or isolated); 2) the primer nucleic acid is immobilized to the solid support and the target nucleic acid is hybridized thereto (this is for detecting the presence of or sequencing a target sequence in a sample); or 3) a double stranded DNA (amplified or isolated) is immobilized through linkage to one predetermined strand; the DNA is denatured to eliminate the duplex and then a high concentration of a complementary primer or DNA with identity upstream from the target site is added and a strand displacement occurs and the primer is hybridized to the immobilized strand.

In the embodiments where the primer nucleic acid is immobilized on the solid support and the target nucleic acid is hybridized thereto, the inclusion of the cleavable linker allows the primer DNA to be immobilized at the 5'-end so that free 3'-OH is available for nucleic acid synthesis (extension) and the sequence of the "hybridized" target DNA can be determined because the hybridized template can be removed by denaturation and the extended DNA products cleaved from the solid support for MALDI-TOF MS. Similarly for 3), the immobilized DNA strand can be elongated when hybridized to the template and cleaved from the support. Thus, Sanger sequencing and primer oligo base extension (PROBE), discussed below, extension reactions can be performed using an immobilized primer of a known, upstream DNA sequence complementary to an invariable region of a target sequence. The nucleic acid from the person is obtained and the DNA sequence of a variable region (deletion, insertion, missense mutation that cause genetic predisposition or diseases, or the presence of viral/bacterial or fungal DNA) not only is detected, but the actual sequence and position of the mutation is also determined.

In other cases, the target DNA must be immobilized and the primer annealed. This requires amplifying a larger DNA based on known sequence and then sequencing the immobilized fragments (i.e., the extended fragments are hybridized but not immobilized to the support as described above). In these cases, it is not desirable to include a linker because the MALDI-TOF spectrum is of the hybridized DNA; it is not necessary to cleave the immobilized template.

Any linker known to those of skill in the art for immobilizing nucleic acids to solid supports may be used herein to link the nucleic acid to a solid support. The preferred linkers herein are the selectively cleavable linkers, particularly those exemplified herein. Other linkers include, acid cleavable linkers, such as bismaleimideothoxy propane, acid-labile trityl linkers.

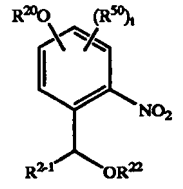
Acid cleavable linkers, photocleavable and heat sensitive linkers may also be used, particularly where it may be necessary to cleave the targeted agent to permit it to be more readily accessible to reaction. Acid cleavable linkers include, but are not limited to, bismaleimideothoxy propane; and adipic acid dihydrazide linkers (see, e.g., Fattom et al. (1992) *Infection & Immun.* 60:584–589) and acid labile transfering conjugates that contain a sufficient portion of transferrin to permit entry into the intracellular transferrin cycling pathway (see, e., Welhöner et al. (1991) *J. Biol. Chem.* 266:4309–4314).

Photocleavable Linkers

Photocleavable linkers are provided. In particular, photocleavable linkers as their phosphoramidite derivatives are provided for use in solid phase synthesis of oligonucleotides. The linkers contain o-nitrobenzyl moieties and phosphate linkages which allow for complete photolytic cleavage of the conjugates within minutes upon UV irradiation. The UV wavelengths used are selected that the irradiation will not damage the oligonucleotides and are preferably about 350–380 nm, more preferably 365 nm. The photocleavable linkers provided herein possess comparable coupling efficiency as compared to commonly used phosphoramidite monomers (see, Sinha et al. (1983) *Tetrahedron Lett.* 24:5843–5846; Sinha et al. (1984) *Nucleic Acids Res.* 12:4539–4557; Beaucage et al. (1993) *Tetrahedron* 49:6123–6194; and Matteucci et al. (1981) *J. Am. Chem. Soc.* 103:3185–3191).

In one embodiment, the photocleavable linkers have formula I:

(I)



where R^{20} is w-(4,4'-dimethoxytrityloxy)alkyl or w-hydroxyalkyl; R^{21} is selected from hydrogen, alkyl, aryl, alkoxycarbonyl, aryloxycarbonyl and carboxy; R^{22} is hydrogen or (dialkylamino)(w-cyanoalkoxy)P-; t is 0–3; and R^{50} is alkyl, alkoxy, aryl or aryloxy.